Jérôme Delon and Ronald N. Germain respond:

In our recent review about the immunological synapse [1], we discussed at some length our conclusion that the *raison d'être* of such a structure was both to

contribute "to stabilizing signal transduction by the TCR" as well as to guide the "polarization of the cell's secretory machinery". Although Davis and van der Merwe agree on the importance of synapse-induced polarization for secretion-based phenomena and antigen-non-specific cell–cell surface protein interactions, they do not see a role for the synapse in prolonging TCR signaling. While these investigators' emphasis on the less well-recognized role of the synapse in polarized intercellular communication is valuable, we think it is inappropriate at this point to dismiss the role of synaptic architecture in facilitating the long-term activity of the TCR.

Several pieces of experimental evidence support the view that some low intensity TCR signal transduction events continue well after the completion of synapse maturation, around 30 minutes or more after the initial T cell–APC contact. This is particularly obvious in single-cell studies of a so-called early readout of T cell activation, the well-defined increase of intracellular calcium (Ca^{2+}) concentration. Following the early peak of Ca^{2+} elevation, one can typically record a plateau corresponding to a Ca^{2+} concentration above the baseline level that is maintained for more than 1 hour [2]. This plateau is considered to be necessary to support the nuclear localization of the transcription factor NF-AT, which plays a major role in the transcription of several cytokine genes [3].

The finding that a sustained Ca^{2+} response exceeding 2 hours in duration is necessary to elicit interleukin-2 production [4,5] is also consistent with the TCR-dependent maintenance of a Ca^{2+} plateau that lasts beyond the first 30 minutes of T cell–APC interaction. It is highly unlikely that the signal maintaining this slightly elevated Ca^{2+} level comes from a different origin other than a low level of TCR signaling. Accessory molecules are not expressed in the APC used in the cited study [2] nor are they known to trigger this kind of signal when bound by their physiological ligands rather than aggregated by antibody-induced cross-linking [6]. And cytokine receptor signaling does not involve a Ca^{2+} rise [7]. Further experimental support for this perspective comes from a report revealing the necessity of prolonged TCR occupancy for cytokine production [8]. In this work, the authors show that adding a blocking antibody specific for MHC class II molecules as late as 1 hour after T cell–APC conjugate formation results in a 70% inhibition of interferon-γ production. These data clearly argue for the necessity of continued TCR signaling well after synapse formation is complete.

In conclusion, we envision a dual role for the immunological synapse: it polarizes the T cell secretory apparatus towards the APC and it also participates in sustained TCR signaling, at a minimum by virtue of the fact that the synapse is the only subcellular region of the T cell where antigen receptors can still be engaged by their ligands. Beyond this, however, the specific organization of the synapse may play a specific role in this late signaling. We have argued in the past that the high local density of TCR and ligand in the opposing membranes of the synapse may facilitate repetitive rebinding of ligands as they dissociate from the TCR, producing an effect simulating high affinity binding [9]. This may be critical for achieving signaling after feedback regulatory processes begin to attenuate the efficacy of TCR function. Furthermore, although Davis and van der Merwe focus on the exclusion of CD45 from the synapse with respect to the role of this phosphatase in activating Src family kinases, the sequestration of tyrosine phosphoproteins away from CD45 in the mature synapse may be key to avoiding the documented negative role of this phosphatase in TCR signal transduction [10]. This could also play a role in permitting low tonic signaling to proceed over a prolonged period. Obviously, only new experiments will ultimately determine whether synaptic organization *per se* has this postulated primary role in facilitating the late TCR signaling that existing experimental evidence clearly shows is crucial to effector gene expression in T cells.

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